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Introduction

The annual plant *Artemisia annua* L. (Sweet wormwood, Asteraceae) is known for its high content of the anti-malaria agent artemisinin and related sesquiterpene lactones, but is also rich in other potential bioactive secondary metabolites such as essential oils, coumarins and flavonoids. In the present study the extracts of the aerial parts of *A. annua* was investigated for their antibacterial and antiparasitic effects *in vitro* and *in vivo* and their possible use as antibacterial and/or antiparasitic additives in poultry feed.

In the work presented here, the focus was :

1) Determination of the MIC values of the extracts for use on *Clostridium perfringens* (CP).
2) Determination of the antiparasitic effect against *Histomonas meleagridis* (protozoan genus) of the extracts.
3) Preliminary identification of antimicrobial and antiparasitic metabolites of *A. annua* extracts/fractions.

Fig. 1. *Artemisia annua*. Photo: K. Grevsen

Antimicrobial and antiparasitic activities of *A. annua* extracts *in vitro* and *in vivo.*

Fig 2. Broiler Ross 308. Photo: http://en.aviagen.com/ross/

Antibacterial tests, in overnight cultures of CP strains, isolated from diseased broilers, showed that *n*-hexane and dichloromethane (DCM) extracts of *A. annua* aerial parts exhibited strong inhibition on CP with mean MIC at 170 and 270 ppm, respectively.

The *n*-hexane extract was tested as a food additive in necrotic enteritis (NE) disease model on broilers to evaluate its effect on the severity of the disease in the broilers. The extract could not totally prevent the development of NE but it decreased the intestinal numbers of CP and the severity of the intestinal lesions ($P < 0.05$). The birds that received the extract also gained more weight than the control animals ($P < 0.05$).

Preliminary studies also showed that the *n*-hexane and DCM extracts have antiparasitic effect against *Histomonas meleagridis*, a parasite causing blackhead disease in poultry. The inhibition of the parasite by the tested extracts indicated a dose dependent inhibition.

Bioactive compounds in *A. annua*

Preliminary bioassay-guided fractionation of the *n*-hexane and DCM extracts by flash column chromatography and reverse phase semi-preparative HPLC revealed the presence of several secondary metabolites in active fractions that can be responsible for the observed bioactivity of the extracts.

The metabolites were identified by GC-MS and/or liquid chromatography multiple-step tandem electrospray mass spectrometry (LC-ESI-MS^n) combined with photodiode array detection (PDA) and evaporative light scattering detection (ELSD). Active fractions contained several compounds that have shown antibacterial effect [1–4] such as borneol, eugenol, linalool, α-pinene, (+)-sabinene, α-terpineol, coumarin, scopoletin, camphor, camphene, quercetin and kaempferol.

The antiparasitic effect can be ascribed to the content of artemisinin and dihydroartemisinin in the extracts but also other compounds such as flavonoids may induce this response. *In vitro* experiments have shown that the flavonoids quercetin, apigenin, luteolin and kaempferol had both individual and synergistic effects against the protozoan *Plasmodium falciparum* [5]

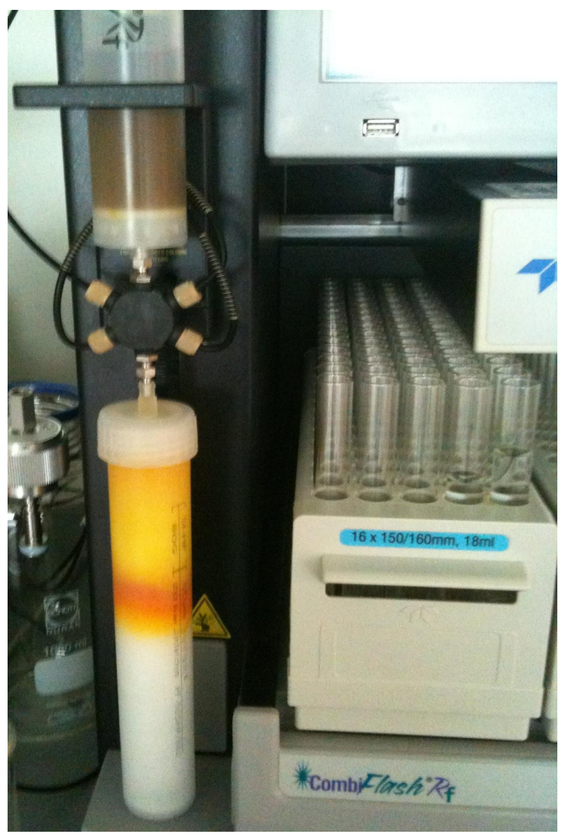

Fig. 3. Fractionation of *n*-hexane extract by flash column chromatography

Conclusions & Perspectives

- In *vitro* experiments with extracts of *A. annua* show antibacterial effect as they inhibit growth of *C. perfringens* at low concentrations and furthermore they also exhibited antiparasitic effect against *Histomonas meleagridis*.
- Analysis of *A. annua* extracts and fractions shows that they contains different groups of natural products, which can explain the antibacterial and antiparasitic activities of A. annua and their potential use as additives in poultry feed.
- *In vivo* testing of the hexane extract also showed promising results and the bioavailability of artemisinin and other bioactive constituents is now being investigated.
- Additional investigations are currently undertaken to identify the compounds responsible for the antibacterial activity against CP.

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